SEQUENCE LISTING

<110> Chemicon International, Inc.
LENG, Jay

<120> PROTEASE SPECIFIC CLEAVABLE LUCIFERASES AND METHODS OF USE THEREOF

<130> CHEM1110

<140> US 09/619,047
<141> 2000-07-18

<160> 29

<170> PatentIn version 3.0

<210> 1
<211> 936
<212> DNA
<213> Renilla reniformis

<220>
<221> CDS
<222> (1)..(936)

<400> 1

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Met Thr Ser Lys Val Tyr Asp Pro Glu Gln Arg Lys Arg Met Ile Thr
1               5                   10                  15

ggt ccg cag tgg tgg gcc aga tgt aaa caa atg aat gtt ctt gat tca       96
Gly Pro Gln Trp Trp Ala Arg Cys Lys Gln Met Asn Val Leu Asp Ser
            20                  25                  30

ttt att aat tat tat gat tca gaa aaa cat gca gaa aat gct gtt att      144
Phe Ile Asn Tyr Tyr Asp Ser Glu Lys His Ala Glu Asn Ala Val Ile
        35                  40                  45

ttt tta cat ggt aac gcg gcc tct tct tat tta tgg cga cat gtt gtg      192
Phe Leu His Gly Asn Ala Ala Ser Ser Tyr Leu Trp Arg His Val Val
    50                  55                  60

cca cat att gag cca gta gcg cgg tgt att ata cca gat ctt att ggt      240
Pro His Ile Glu Pro Val Ala Arg Cys Ile Ile Pro Asp Leu Ile Gly
65                  70                  75                  80

atg ggc aaa tca ggc aaa tct ggt aat ggt tct tat agg tta ctt gat      288
Met Gly Lys Ser Gly Lys Ser Gly Asn Gly Ser Tyr Arg Leu Leu Asp
                85                  90                  95

cat tac aaa tat ctt act gca tgg ttt gaa ctt ctt aat tta cca aag      336
His Tyr Lys Tyr Leu Thr Ala Trp Phe Glu Leu Leu Asn Leu Pro Lys
            100                 105                 110

aag atc att ttt gtc ggc cat gat tgg ggt gct tgt ttg gca ttt cat      384
Lys Ile Ile Phe Val Gly His Asp Trp Gly Ala Cys Leu Ala Phe His
        115                 120                 125

tat agc tat gag cat caa gat aag atc aaa gca ata gtt cac gct gaa      432
Tyr Ser Tyr Glu His Gln Asp Lys Ile Lys Ala Ile Val His Ala Glu
    130                 135                 140
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agt gta gta gat gtg att gaa tca tgg gat gaa tgg cct gat att gaa      480
Ser Val Val Asp Val Ile Glu Ser Trp Asp Glu Trp Pro Asp Ile Glu
145                 150                 155                 160

gaa gat att gcg ttg atc aaa tct gaa gaa gga gaa aaa atg gtt ttg      528
Glu Asp Ile Ala Leu Ile Lys Ser Glu Glu Gly Glu Lys Met Val Leu
                165                 170                 175

gag aat aac ttc ttc gtg gaa acc atg ttg cca tca aaa atc atg aga      576
Glu Asn Asn Phe Phe Val Glu Thr Met Leu Pro Ser Lys Ile Met Arg
            180                 185                 190

aag tta gaa cca gaa gaa ttt gca gca tat ctt gaa cca ttc aaa gag      624
Lys Leu Glu Pro Glu Glu Phe Ala Ala Tyr Leu Glu Pro Phe Lys Glu
        195                 200                 205

aaa ggt gaa gtt cgt cgt cca aca tta tca tgg cct cgt gaa atc ccg      672
Lys Gly Glu Val Arg Arg Pro Thr Leu Ser Trp Pro Arg Glu Ile Pro
    210                 215                 220

tta gta aaa ggt ggt aaa cct gac gtt gta caa att gtt agg aat tat      720
Leu Val Lys Gly Gly Lys Pro Asp Val Val Gln Ile Val Arg Asn Tyr
225                 230                 235                 240

aat gct tat cta cgt gca agt gat gat tta cca aaa atg ttt att gaa      768
Asn Ala Tyr Leu Arg Ala Ser Asp Asp Leu Pro Lys Met Phe Ile Glu
                245                 250                 255

tcg gat cca gga ttc ttt tcc aat gct att gtt gaa ggc gcc aag aag      816
Ser Asp Pro Gly Phe Phe Ser Asn Ala Ile Val Glu Gly Ala Lys Lys
            260                 265                 270

ttt cct aat act gaa ttt gtc aaa gta aaa ggt ctt cat ttt tcg caa      864
Phe Pro Asn Thr Glu Phe Val Lys Val Lys Gly Leu His Phe Ser Gln
        275                 280                 285

gaa gat gca cct gat gaa atg gga aaa tat atc aaa tcg ttc gtt gag      912
Glu Asp Ala Pro Asp Glu Met Gly Lys Tyr Ile Lys Ser Phe Val Glu
    290                 295                 300

cga gtt ctc aaa aat gaa caa taa                                     936
Arg Val Leu Lys Asn Glu Gln
305                 310
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<210> 2
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<213> Renilla reniformis

<400> 2

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Met Thr Ser Lys Val Tyr Asp Pro Glu Gln Arg Lys Arg Met Ile Thr
1               5                   10                  15

Gly Pro Gln Trp Trp Ala Arg Cys Lys Gln Met Asn Val Leu Asp Ser
            20                  25                  30
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```
Phe Ile Asn Tyr Tyr Asp Ser Glu Lys His Ala Glu Asn Ala Val Ile
        35                  40                  45

Phe Leu His Gly Asn Ala Ala Ser Ser Tyr Leu Trp Arg His Val Val
    50                  55                  60

Pro His Ile Glu Pro Val Ala Arg Cys Ile Ile Pro Asp Leu Ile Gly
65                  70                  75                  80

Met Gly Lys Ser Gly Lys Ser Gly Asn Gly Ser Tyr Arg Leu Leu Asp
                85                  90                  95

His Tyr Lys Tyr Leu Thr Ala Trp Phe Glu Leu Leu Asn Leu Pro Lys
            100                 105                 110

Lys Ile Ile Phe Val Gly His Asp Trp Gly Ala Cys Leu Ala Phe His
        115                 120                 125

Tyr Ser Tyr Glu His Gln Asp Lys Ile Lys Ala Ile Val His Ala Glu
    130                 135                 140

Ser Val Val Asp Val Ile Glu Ser Trp Asp Glu Trp Pro Asp Ile Glu
145                 150                 155                 160

Glu Asp Ile Ala Leu Ile Lys Ser Glu Glu Gly Glu Lys Met Val Leu
                165                 170                 175

Glu Asn Asn Phe Phe Val Glu Thr Met Leu Pro Ser Lys Ile Met Arg
            180                 185                 190

Lys Leu Glu Pro Glu Glu Phe Ala Ala Tyr Leu Glu Pro Phe Lys Glu
        195                 200                 205

Lys Gly Glu Val Arg Arg Pro Thr Leu Ser Trp Pro Arg Glu Ile Pro
    210                 215                 220

Leu Val Lys Gly Gly Lys Pro Asp Val Val Gln Ile Val Arg Asn Tyr
225                 230                 235                 240

Asn Ala Tyr Leu Arg Ala Ser Asp Asp Leu Pro Lys Met Phe Ile Glu
                245                 250                 255

Ser Asp Pro Gly Phe Phe Ser Asn Ala Ile Val Glu Gly Ala Lys Lys
            260                 265                 270

Phe Pro Asn Thr Glu Phe Val Lys Val Lys Gly Leu His Phe Ser Gln
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        275                 280                 285

Glu Asp Ala Pro Asp Glu Met Gly Lys Tyr Ile Lys Ser Phe Val Glu
    290                 295                 300

Arg Val Leu Lys Asn Glu Gln
305                 310
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<210> 3
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<212> DNA
<213> Renilla reniformis (mutated sequence)

<220>
<221> CDS
<222> (1)..(936)

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atg act tcg aaa gtt tat gat cca gaa caa agg aaa cgg atg ata act       48
Met Thr Ser Lys Val Tyr Asp Pro Glu Gln Arg Lys Arg Met Ile Thr
1               5                   10                  15

ggt ccg cag tgg tgg gcc aga tgt aaa caa atg aat gtt ctt gat tca       96
Gly Pro Gln Trp Trp Ala Arg Cys Lys Gln Met Asn Val Leu Asp Ser
            20                  25                  30

ttt att aat tat tat gat tca gaa aaa cat gca gaa aat gct gtt att      144
Phe Ile Asn Tyr Tyr Asp Ser Glu Lys His Ala Glu Asn Ala Val Ile
        35                  40                  45

ttt tta cat ggt aac gcg gcc tct tct tat tta tgg cga cat gtt gtg      192
Phe Leu His Gly Asn Ala Ala Ser Ser Tyr Leu Trp Arg His Val Val
    50                  55                  60

cca cat att gag cca gta gcg cgg tgt att ata cca gat ctt att ggt      240
Pro His Ile Glu Pro Val Ala Arg Cys Ile Ile Pro Asp Leu Ile Gly
65                  70                  75                  80

atg ggc aaa tca ggc aaa tct ggt aat ggt tct tat agg tta ctt gat      288
Met Gly Lys Ser Gly Lys Ser Gly Asn Gly Ser Tyr Arg Leu Leu Asp
                85                  90                  95

cat tac aaa tat ctt act gca tgg ttt gaa ctt ctt aat tta cca aag      336
His Tyr Lys Tyr Leu Thr Ala Trp Phe Glu Leu Leu Asn Leu Pro Lys
            100                 105                 110

aag atc att ttt gtc ggc cat gat tgg ggt gct tgt ttg gca ttt cat      384
Lys Ile Ile Phe Val Gly His Asp Trp Gly Ala Cys Leu Ala Phe His
        115                 120                 125

tat agc tat gag cat caa gat aag atc aaa gca ata gtt cac gct gaa      432
Tyr Ser Tyr Glu His Gln Asp Lys Ile Lys Ala Ile Val His Ala Glu
    130                 135                 140

agt gta gta gat gtg att gaa tca tgg gat gaa tgg cct gat att gaa      480
Ser Val Val Asp Val Ile Glu Ser Trp Asp Glu Trp Pro Asp Ile Glu
145                 150                 155                 160
```

```
gaa gat att gcg ttg atc aaa tct gaa gaa gga gaa aaa atg gtt ttg      528
Glu Asp Ile Ala Leu Ile Lys Ser Glu Glu Gly Glu Lys Met Val Leu
                165                 170                 175

gag aat aac ttc ttc gtg gaa acc atg ttg cca tca aaa atc atg aga      576
Glu Asn Asn Phe Phe Val Glu Thr Met Leu Pro Ser Lys Ile Met Arg
            180                 185                 190

aag tta gaa cca gac gaa gtt gac gca tat ctt gaa cca ttc aaa gag      624
Lys Leu Glu Pro Asp Glu Val Asp Ala Tyr Leu Glu Pro Phe Lys Glu
        195                 200                 205

aaa ggt gaa gtt cgt cgt cca aca tta tca tgg cct cgt gaa atc ccg      672
Lys Gly Glu Val Arg Arg Pro Thr Leu Ser Trp Pro Arg Glu Ile Pro
    210                 215                 220

tta gta aaa ggt ggt aaa cct gac gtt gta caa att gtt agg aat tat      720
Leu Val Lys Gly Gly Lys Pro Asp Val Val Gln Ile Val Arg Asn Tyr
225                 230                 235                 240

aat gct tat cta cgt gca agt gat gat tta cca aaa atg ttt att gaa      768
Asn Ala Tyr Leu Arg Ala Ser Asp Asp Leu Pro Lys Met Phe Ile Glu
                245                 250                 255

tcg gat cca gga ttc ttt tcc aat gct att gtt gaa ggc gcc aag aag      816
Ser Asp Pro Gly Phe Phe Ser Asn Ala Ile Val Glu Gly Ala Lys Lys
            260                 265                 270

ttt cct aat act gaa ttt gtc aaa gta aaa ggt ctt cat ttt tcg caa      864
Phe Pro Asn Thr Glu Phe Val Lys Val Lys Gly Leu His Phe Ser Gln
        275                 280                 285

gaa gat gca cct gat gaa atg gga aaa tat atc aaa tcg ttc gtt gag      912
Glu Asp Ala Pro Asp Glu Met Gly Lys Tyr Ile Lys Ser Phe Val Glu
    290                 295                 300

cga gtt ctc aaa aat gaa caa taa                                     936
Arg Val Leu Lys Asn Glu Gln
305                 310
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<210> 4
<211> 311
<212> PRT
<213> Renilla reniformis (mutated sequence)

<400> 4

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Gly Pro Gln Trp Trp Ala Arg Cys Lys Gln Met Asn Val Leu Asp Ser
            20                  25                  30

Phe Ile Asn Tyr Tyr Asp Ser Glu Lys His Ala Glu Asn Ala Val Ile
        35                  40                  45

Phe Leu His Gly Asn Ala Ala Ser Ser Tyr Leu Trp Arg His Val Val
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```
    50                  55                  60

Pro His Ile Glu Pro Val Ala Arg Cys Ile Ile Pro Asp Leu Ile Gly
65                  70                  75                  80

Met Gly Lys Ser Gly Lys Ser Gly Asn Gly Ser Tyr Arg Leu Leu Asp
                85                  90                  95

His Tyr Lys Tyr Leu Thr Ala Trp Phe Glu Leu Leu Asn Leu Pro Lys
            100                 105                 110

Lys Ile Ile Phe Val Gly His Asp Trp Gly Ala Cys Leu Ala Phe His
        115                 120                 125

Tyr Ser Tyr Glu His Gln Asp Lys Ile Lys Ala Ile Val His Ala Glu
    130                 135                 140

Ser Val Val Asp Val Ile Glu Ser Trp Asp Glu Trp Pro Asp Ile Glu
145                 150                 155                 160

Glu Asp Ile Ala Leu Ile Lys Ser Glu Glu Gly Glu Lys Met Val Leu
                165                 170                 175

Glu Asn Asn Phe Phe Val Glu Thr Met Leu Pro Ser Lys Ile Met Arg
            180                 185                 190

Lys Leu Glu Pro Asp Glu Val Asp Ala Tyr Leu Glu Pro Phe Lys Glu
        195                 200                 205

Lys Gly Glu Val Arg Arg Pro Thr Leu Ser Trp Pro Arg Glu Ile Pro
    210                 215                 220

Leu Val Lys Gly Gly Lys Pro Asp Val Val Gln Ile Val Arg Asn Tyr
225                 230                 235                 240

Asn Ala Tyr Leu Arg Ala Ser Asp Asp Leu Pro Lys Met Phe Ile Glu
                245                 250                 255

Ser Asp Pro Gly Phe Phe Ser Asn Ala Ile Val Glu Gly Ala Lys Lys
            260                 265                 270

Phe Pro Asn Thr Glu Phe Val Lys Val Lys Gly Leu His Phe Ser Gln
        275                 280                 285

Glu Asp Ala Pro Asp Glu Met Gly Lys Tyr Ile Lys Ser Phe Val Glu
    290                 295                 300
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Arg Val Leu Lys Asn Glu Gln
305 310

<210> 5
<211> 8
<212> PRT
<213> Artificial

<220>
<223> Description of Artificial Sequence: Protease recognition sequence

<400> 5

Ser Gln Asn Tyr Pro Ile Val Gln
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<210> 6
<211> 10
<212> PRT
<213> Artificial

<220>
<223> Description of Artificial Sequence: Protease recognition sequence

<400> 6

Lys Ala Arg Val Leu Ala Glu Ala Met Ser
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<210> 7
<211> 10
<212> PRT
<213> Artificial

<220>
<223> Description of Artificial Sequence: Protease recognition sequence

<400> 7

Pro Ser Pro Arg Glu Gly Lys Arg Ser Tyr
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<210> 8
<211> 5
<212> PRT
<213> Artificial

<220>
<223> Description of Artificial Sequence: Protease recognition sequence

<400> 8

Tyr Val Ala Asp Gly
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<210> 9
<211> 8
<212> PRT

<213> Artificial

<220>
<223> Description of Artificial Sequence: Protease recognition sequence

<400> 9

Met Phe Gly Gly Ala Lys Lys Arg
1 5

<210> 10
<211> 10
<212> PRT
<213> Artificial

<220>
<223> Description of Artificial Sequence: Protease recognition sequence

<400> 10

Gly Val Val Asn Ala Ser Ser Arg Leu Ala
1 5 10

<210> 11
<211> 9
<212> PRT
<213> ARTIFICIAL

<220>
<223> Description of Artificial Sequence: Protease recognition sequence

<400> 11

Leu Ile Ala Tyr Leu Lys Lys Ala Thr
1 5

<210> 12
<211> 7
<212> PRT
<213> ARTIFICIAL

<220>
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Val Lys Met Asp Ala Glu Phe
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<210> 13
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Description of Artificial Sequence: Protease recognition sequence

<400> 13

Phe Leu Ala Glu Gly Gly Gly Val Arg Gly Pro Arg Val Val Glu Arg
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His

<210> 14
<211> 13
<212> PRT
<213> Artificial

<220>
<223> Description of Artificial Sequence: Protease recognition sequence

<400> 14

Asp Arg Val Tyr Ile His Pro Phe His Leu Val Ile His
1 5 10

<210> 15
<211> 8
<212> PRT
<213> Artificial

<220>
<223> Description of Artificial Sequence: Protease recognition sequence

<400> 15

Lys Pro Ala Leu Phe Phe Arg Leu
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<210> 16
<211> 4
<212> PRT
<213> Artificial

<220>
<223> Description of Artificial Sequence: Protease recognition sequence

<400> 16

Ile Glu Pro Asp
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<210> 17
<211> 4
<212> PRT
<213> Artificial

<220>
<223> Description of Artificial Sequence: Protease recognition sequence

<400> 17

Asp Glu Thr Asp
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<210> 18
<211> 4
<212> PRT
<213> Artificial

<220>
<223> Description of Artificial Sequence: Protease recognition sequence

<400> 18

Trp Glu His Asp
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<210> 19
<211> 4
<212> PRT
<213> Artificial

<220>
<223> Description of Artificial Sequence: Protease recognition sequence

<400> 19

Tyr Val Ala Asp
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<210> 20
<211> 4
<212> PRT
<213> Artificial

<220>
<223> Description of Artificial Sequence: Protease recognition sequence

<400> 20

Asp Glu His Asp
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<210> 21
<211> 4
<212> PRT
<213> Artificial

<220>
<223> Description of Artificial Sequence: Protease recognition sequence

<400> 21

Asp Glu Val Asp
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<210> 22
<211> 4
<212> PRT
<213> Artificial

<220>
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<220>
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<222> (1)..(1)
<223> Xaa is Trp or Ley

<400> 22

Xaa Glu His Asp
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<211> 4
<212> PRT
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<220>
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<222> (3)..(3)
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<400> 23

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<211> 4
<212> PRT
<213> Artificial

<220>
<223> Description of Artificial Sequence: Protease recognition sequence

<400> 24

Leu Glu Thr Asp
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<211> 4
<212> PRT
<213> Artificial

<220>
<223> Description of Artificial Sequence: Protease recognition sequence

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<210> 26
<211> 4
<212> PRT
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<223> Xaa is any amino acid

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<210> 27
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<220>
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<400> 27

Arg Pro Leu Gly Ile Ile Gly Gly
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<210> 28
<211> 3
<212> PRT
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<220>
<223> Description of Artificial Sequence: Protease recognition sequence

<400> 28

Glu Gly Arg
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<210> 29
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<212> PRT
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<220>
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<400> 29

Val Leu Lys
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